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Clustering of C_2–H_2 Zinc Finger Motif Sequences within Telomeric and Fragile Site Regions of Human Chromosomes

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Ninety-three phage clones identified by hybridization with a C_2–H_2 zinc finger sequence probe have been grouped into 23 genetic loci. Partial sequencing verified that each locus belonged to the zinc finger family. Oligonucleotide primer pairs were developed from these sequences to serve as STS markers for these loci. One or more clones from each locus was mapped onto human metaphase chromosomes by fluorescence *in situ* hybridization. Several loci map to identical chromosomal regions, indicating the possible presence of multigene clusters. Zinc finger loci were found to reside predominantly either in telomeric regions or in chromosomal bands known to exhibit chromosome fragility. Chromosome 19 carries a disproportionate fraction (10 of 23) of the mapped zinc finger loci.

INTRODUCTION

Proteins containing the zinc finger motif include several regulatory proteins with developmentally important functions (e.g., Krüppel, Rosenberg *et al.*, 1986), transcription factors (e.g., SP1, Kadonga *et al.*, 1987), early response products to growth factors (Chavrier *et al.*, 1988), and possible oncogenes or growth suppressors (GLI, Kinzler *et al.*, 1988; Wilms Tumor, Call *et al.*, 1990). These varied and important functions suggest that proteins of the zinc finger class are involved in many critical processes in the regulation of gene expression. The DNA binding domain of zinc finger proteins consists of tandem arrays of two or more zinc fingers; each finger is composed of approximately 30 amino acids with a consensus sequence of Cys–X2-4–Cys–X3–Phe–X5–Leu–*X*2–His–*X*3–4–His, where *X* indicates amino acids that are less well conserved. A tetrahedrally coordinated zinc ion is bound by the conserved Cys and His residues (Miller *et al.*, 1985; Klug and Rhodes, 1987), serving as the basis for finger folding to bring key amino acids into close proximity with nucleotide residues in the target sequences. The amino acids joining adjacent fingers, most commonly TGEKPYE/K (H/C link), may play a key role in alignment of finger domains within the major groove of the DNA helix (Pavletich and Pabo, 1991). The amino acids in the H/C link region are more highly conserved than those within the fingers except for the Cys and His residues that define the motif (Schuh *et al.*, 1986); the link region consensus sequence has been used to isolate C_2–H_2 zinc finger genes from Xenopus (Nietfeld *et al.*, 1989; Ruiz i Altaba *et al.*, 1987) and human (Bellefroid *et al.*, 1989; Thiesen, 1990) cDNA libraries and from human (Bray *et al.*, 1991; Hoovers *et al.*, 1992) and mouse (Chowdhury *et al.*, 1987) genomic libraries.

It has been estimated that there may be as many as 300–400 zinc finger proteins encoded in the genomes of vertebrates (Bellefroid *et al.*, 1989; Bray and Thiesen, 1990). Various members of the mouse and human zinc finger protein multigene families have been identified, and several of them have been localized on chromosomes (reviewed by Ashworth and Denny, 1991). In the mouse, a class of zinc finger encoding genes related to the Drosophila Krüppel gene has been described, and four of them have been mapped, Zip-1 and Zip-4 to Chr 8 and Zip-2 and Zip-3 to Chr 11, suggesting two gene complexes on Chr 8 and Chr 11 (Chowdhury *et al.*, 1989; Ashworth *et al.*, 1989; Nadeau *et al.*, 1990). Further zinc finger genes identified in the mouse include the ecotropic viral integration site 1 (Evi-1) on Chr 3 (Morishita *et al.*, 1988), the Zfy-1 and Zfy-2 genes on the Y chromosome (Page *et al.*, 1987), the gene for the transcription factor SP1 on the distal end of Chr 15 (Saffer *et al.*, 1990), and the Krox gene family (Chavrier *et al.*, 1988). The human zinc finger protein genes that have been mapped include the genes for glioblastoma (GL1) on Chr 12q13.4–q14.3, the early growth response gene 1 (EGR1) on Chr 5q23–q31, the zinc finger protein 3 (ZFP3) on Chr 17p12–p17, the zinc finger proteins 1 to 8, 24, and 35 (ZNF1 to ZNF8, ZNF24, and ZNF35) on Chr 8, 2, 5, 5p14–p13, 5q12–q13, Xq13–q21.1, 8q24, 20q13, 18q12,

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and 3p22–p21, respectively, ZFX and ZFY on Xp22.1–p21.3 and Yp11.3, respectively, the GLI3 protein on Chr 7p13, as well as the candidate for the Wilms tumor gene on Chr 11p13 (summarized in McAlpine *et al.*, 1990).

To determine the size, diversity, and chromosomal distribution of zinc finger encoding genes in the human genome, we have isolated and analyzed a number of clones from a human genomic DNA library. Clones were selected to contain sequences homologous to the C_2–H_2 subtype of the zinc finger motif, like those found in the Drosophila gene Krüppel. We have previously described the general approach to the isolation and characterization of zinc finger genomic clones, presenting partial sequence and mapping data for four zinc finger genes (Bray *et al.*, 1991). Here we report the characterization of 19 additional groups of genomic clones and their chromosomal localization.

MATERIALS AND METHODS

Clone isolation. A genomic library of human placenta DNA (provided by P. Leder) was screened with a degenerate oligonucleotide probe that represents the conserved H/C link region of C_2-H_2 finger proteins (Schuh *et al.*, 1986). The probe sequence was:

G	AAG	CAC	CAG	CGG	ACG	CAC	ACG	GGG	GAG	AAG	CCC	TAC	CA	
	C		T	A	TT			A						
K/N	H	L/Q	R	I/M/T	H	T	G	E	K	P	Y			

Approximately 5×10^5 phage were screened by hybridization in a solution containing 5× SSC, 5× Denhardt's solution, 50 m*M* sodium phosphate (pH 6.8), 1 m*M* sodium pyrophosphate, 50 μg of yeast RNA per ml, 10% formamide, and ^{32}P-labeled oligonucleotide probe (1.5×10^6 cpm, 175 fmol/ml). Filters were hybridized for 16 h at 42°C, washed three times in 0.2× SSC, 0.1% SDS at 23°C for 10 min per wash and once at 39°C for 2 min, and then subjected to autoradiography.

Characterization of clones. Approximately 600 plaques were detected, 238 were picked, and 208 of these were plaque purified. Clones were sorted as previously described (Bray *et al.*, 1991). Nucleotide sequence was determined for portions of zinc finger encoding regions of randomly chosen clones, and locus-specific oligonucleotide probes were synthesized. Dot blots of DNAs of all clones were hybridized with these oligonucleotide probes at high stringency. All clones that hybridized to a locus-specific oligonucleotide were used as templates for PCR amplification with oligonucleotide primer pairs derived from the nucleotide sequences of the prototype clone for that locus. PCR reactions were carried out using the AmpliTaq reagent kit (Cetus) for 25 cycles with denaturation at 95°C for 1 min, annealing at T_a (see Table 1) for 1 min, and synthesis at 72°C for 1 min. Phage supernatant (0.5 μl) was used as a source of DNA template in each 20-μl reaction. Only phage clones that yielded PCR fragments of the predicted size were considered to belong to a given group (i.e., locus). One or more clones from which a finger encoding nucleotide sequence was derived were selected for preparation of DNA for hybridization to metaphase chromosomes.

Other DNA probes. The following probes were used for cohybridization experiments: the satellite III DNA probe pUC1.77 (D1Z1) specific for the heterochromatic region 1q12 (Cooke and Hindley, 1979) kindly sent by Howard Cooke (Edinburgh), the alphoid probe D10Z1 labeling the centromere of chromosome 10 (Devilee *et al.*, 1988) kindly provided by Thomas Cremer (Heidelberg), the alphoid probe pSE16 (D16Z2) staining the centromeric region of human chromosome 16 (Greig *et al.*, 1989) kindly supplied by Huntington Willard (Stanford), the DNA library pBS18 derived from sorted and amplified human chromosome 18 DNA sequences (Collins *et al.*, 1991) generously provided by Joe Gray (Livermore), and the *Alu*-PCR products of a monochromosomal hybrid line (5HL94) containing specifically amplified sequences of human chromosome 19 (Ledbetter *et al.*, 1990) kindly provided by Susan Ledbetter (Houston).

In situ hybridization to metaphase chromosomes. Human metaphase chromosome spreads were prepared from cultured lymphocytes using standard methods of colcemid arrest, hypotonic treatment, and

TABLE 1

STS Parameters for Each Locus

Gene	Primer	T_a	Frag size
ZNF7[a]	AGGGCTCCACACCTTATTCAGCATCA	57	400
	CCATATTCGTAAAACCACTGGGCCC		
ZNF19[a]	ACGCTGATGTTGGCTTAGATGGGAA	56	172
	GAGAATCCACACTGGGGAGAAACCC		
ZNF22[a]	CGGTGTTTCAGCCAGAGCTCCCAC	57	247
	CCTATACTTAACGGAGGCCAGCCAC		
ZNF44[a]	TGGGAAATTGTTATCTCATCGCT	53	151
	GGGTTTCTCTCCAGTGTGAGTCCT		
ZNF47	AGGGGAGAACTTCTCACAGCACTGC	61	215
	CAAGAACTCCTCCCTCACTGTGCAC		
ZNF48[a]	GATCTGGTGAAACACCAGCGG	57	135
	GCTCTCTATAGGCTTCTCCCCACTAT		
ZNF49[a]	GGATCTCTCACTGAAGCCTCG	55	252
	CACCTGATTCAGCATCAGACAAT		
ZNF50[a]	TGCAAGGTCGACGGTTATCGATAAGCTT	61	249
	CAGCAGATCAAATTTGTCTTCTGAAGG		
ZNF51[a]	CCTCCGTGCCCATGTGCTTATCCAC	54	147
	CTCAAGAGGCTTACGTACATGG		
ZNF52[a]	AAGCTTTTGTCAGAGCCCAT	55	285
	AGAGATGAGCTATGAGCAAAGGCTTT		
	CACTCAGAATGAAACATGGG	55	650
	TGCTCACTAAGGTGTGCTTG		
ZNF53[b]	GCCTTCAGTCACAAGGAA	42	122
	CTTTGATGTCTAATGAGA		
ZNF54[a,b]	GCCTCAATCCTTCAAATGCATGCTG	53	89
	CTAGAGGATCTGAAGGCTTTCCCA		
ZNF55[a]	AACCCTATACATGTAAACAGTGTGGG	51	118
	AAAGCCTTTCCCACATATCTTACAT		
ZNF56[a]	CTTCACCACAGTCTGACAACTACC	48	115
	TTTTCCAGTATGTCGTATCTTAGGTC		
ZNF57[a]	CCATATGAATGCACCAAGTCGGAC	54	119
	AAATGCAGGCCTGCTTACATTCC		
ZNF58[a]	CTGCAGCCCACTTCCAGTTCCCTTC	54	219
	TTCTTCACTGTGAGTCCTTTCATGGC		
ZNF61[a]	TCAGCCACAGCTCCTCACTCAGCC	60	184
	GACGAGCTGTGACTGAATGCCTTGC		
ZNF62[a]	TTCCTCCCAGAGGCAGGACTTG	60	154
	GTGGATGCGAACGTGGCTCTT		
ZNF64[a]	GTGTGGAGAGGCATTCATTCGAAGC	53	113
	TTCTATTGGAACAGAATGCTCTCCC		
ZNF65[a]	AAGAGCTTCACGCAGAGCTCGCACC	61	116
	TGCACCAGGTTGGAGCTCCAGCTGAA		
ZNF66[a]	GCTTTTGCCACATTCTTCACATT	50	140
	ATGTGTAGAAAGGGAAGAAAGGT		
ZNF67	ATCTGGTCCTCAGGCCTTACTGAAC	52	136
	GTATCCTCTTATGTCTAGTTAGGGA		

Note. The actual primer sequence, not the complements for primers of the opposite orientation, is given for each primer. Some primers are derived from sequences not shown in Fig. 1. T_a is the annealing temperature as estimated using the Oligo program (National Biosciences).

[a] These primers produce fragments of the size predicted from the nucleotide sequences using genomic DNA template as well as phage clone DNA.

[b] The phage clone DNA hybridizes to two sites on the chromosome (ZNF53A/B or ANF54A/B), and it is not known which of the sites, or both, corresponds to the template location for PCR amplification with the primer pairs.

TABLE 2

Summary of the Mapping Methods and Results

Locus	Genomic clone	Map position	Cohybridization	Banding	Chromosomal region
ZNF7	17, 38	8qter	pBS8, Chr library	DAPI-B, *Alu*	Telomere
ZNF19	114	16q22	ND	DAPI-B, *Alu*	FRA16B/FRA16C
ZNF22	27, 30	10cen(q11)	D10Z1, alphoid	DAPI-B	Centromeric, proximal to FRA10C
ZNF44	84	16p11	pSE16, alphoid	DAPI-S, *Alu*	Proximal to FRA16E
ZNF47	56	18qter	ND	DAPI-S	Telomere
ZNF48	58, 74	16p11	pSE16, alphoid	DAPI-B	Proximal to FRA16E
ZNF49	63	1p34	pUC1.77, satellite II	DAPI-B	Proximal to FRA1A, distal to FRA1B
ZNF50	65	19qter	Chr 19–*Alu*-PCR	DAPI-S	Telomere, FRA19A
ZNF51	21, 166, 203	3qter	ND	DAPI-B, *Alu*	Telomere
ZNF52	7, 10, 14, 208	3p21.3	ND	DAPI-B	Distal to FRA3B
ZNF53	111	19pter/qmid	ND	DAPI-S, *Alu*	Telomere, FRA19B/FRA19A
ZNF54	39	19pter/1pdis	Chr 19–*Alu*-PCR	DAPI-S, *Alu*	Telomere, FRA19B
ZNF55	205	19pter	Chr 19–*Alu*-PCR	DAPI-S, *Alu*	Telomere, FRA19B
ZNF56	221	19p13.1	Chr 19–*Alu*-PCR	DAPI-S, *Alu*	—
ZNF57	101	19pter	ND	DAPI-S	Telomere, FRA19B
ZNF58	8	19p13.2	Chr 19–*Alu*-PCR	DAPI-S, *Alu*	FRA19B
ZNF60	144	1pter	pUC1.77, satellite III	DAPI-B	Telomere, FRA1A
ZNF61	32	19qter	Chr 19–*Alu*-PCR	DAPI-S, *Alu*	Telomere, FRA19A
ZNF62	44	17p12	ND	DAPI-B	FRA17A
ZNF64	13, 236	3p21.3	ND	DAPI-B	Distal to FRA3B
ZNF65	161	16p11.2	ND	DAPI-B	Proximal to FRA16E
ZNF66	60, 94	19p13.1	ND	DAPI-B	—
ZNF67	102	19p13.1	ND	DAPI-B	—

Note. Under Locus is listed the assigned gene numbers. Genomic clone indicates which clones were used as probes. Map position refers to the chromosome band location with secondary sites of hybridization listed for ZNF53 and ZNF54. Cohybridization and banding list the methods used for chromosome identification and band identification. Chromosomal region identifies the loci that are localized either at telomeres or close to fragile sites.

methanol/acidic acid fixation. Phage DNA probes were labeled with biotin or digoxigenin by nick translation as described previously (Lichter *et al.*, 1990b) and chromosomal *in situ* suppression (CISS) hybridization was carried out as reported elsewhere (Lichter *et al.*, 1988, 1990b). Briefly, 40 to 80 ng of labeled probe was coprecipitated with 2 to 3 μg of human competitor DNA (total genomic or the C_0t 1 fraction of human DNA) and 7 to 8 μg of salmon sperm DNA and hybridized in a volume of 10 μl of 50% formamide, 1× SSC, and 10% dextran sulfate to denatured metaphase chromosome spreads after the probe had been denaturated (5 min, 75°C) and preannealed (37°C) for 5 to 10 min. In the first set of experiments, biotinylated DNAs were used in single probe hybridizations and detected via avidin-conjugated FITC. Chromosomes were counterstained with propidium iodide and simultaneously banded with DAPI (4',6'-diamidine-2-phenylindole-dihydrochloride).

For confirmation of chromosome assignment, several biotinylated probes containing zinc finger encoding sequences were cohybridized with digoxigenin-labeled DNA probes or probe sets specific for a particular human chromosome (see Table 2). When probe sets such as chromosome-specific DNA libraries (i.e., containing repetitive DNA sequences) were used to tag a chromosome, the probe set was coprecipitated with the DNAs of the hybridization mixture, and the amount of competitor DNA was increased to 4 μg. For cohybridization of the *Alu*-PCR products from a monochromosomal human/rodent somatic cell hybrid containing amplified sequences of human chromosome 19, competitor and salmon sperm carrier DNA were substituted with 10 μg of C_0t 1 DNA as previously reported (Lichter *et al.*, 1990a). When chromosome-specific repetitive DNAs were used for tagging, 40 ng of digoxigenin-labeled probe was combined with 2 μg of salmon sperm DNA in a 4-μl hybridization cocktail (see above), denatured (5 min, 75°C), chilled on ice for 3 min, and combined with the standard hybridization mixture containing the preannealed biotinylated probe before application to the chromosome slides. In dual label experiments, the differentially labeled probes were detected as described (Lichter *et al.*, 1990b) via FITC and Texas Red conjugated proteins, respectively, and the chromosomes were banded with DAPI. When a cooled CCD camera (Photometrics) was used for imaging DAPI and FITC signals, gray scale images were acquired separately and pseudocolored and imaged as described elsewhere (Ried *et al.*, 1992).

Mapping of the DNA probes along the chromosomes in relation to chromosomal bands requires a reliable banding procedure. Although DAPI banding is generally sufficient for most band assignments, the bands cannot always be obtained after DAPI staining on all chromosomes. Therefore, we also used a banding generated by repetitive *Alu*-DNA sequences, which is similar to an R-banding pattern. This was achieved by cohybridization with cloned *Alu*-DNA elements as previously reported (Lichter *et al.*, 1991, 1990b). Occasionally, the differential staining of chromosomes with propidium iodide produced an R-banding pattern that could be used to confirm the band assignment.

RESULTS

Clone Isolation and Characterization

The screening of a human genomic library with the H/C link probe led to the identification of 600 hybridization positive phage (from about 2.5 genome equivalents), 208 of which were plaque purified. Ninety-three of these were grouped initially into 23 loci (Table 3) by hybridization with locus-specific oligonucleotides. Representative clones were chosen for each locus, and a partial nucleic acid sequence in finger motif encoding regions was obtained for each clone. Zinc fingers composed of approximately 28 amino acids have been identified for each of the loci; they show high homology with the previously

TABLE 3

Summary of the Characterization of Human Zinc Finger Loci.

	Clone characterization				
Loci	No. clones (min)[a]	No. oligo hybrid[b]	No. PCR sets[c]	No. FGRs[d]	Genbank Accession No.
ZNF7[e]	6 (6)	2	1	5	M77170
ZNF19[e]	1	1	—	3	M77171
ZNF22[e]	4 (2)	2	2	2	M77172
ZNF44[e]	2 (1)	2	1	2	M77173
ZNF47	5 (4)	2	1	2	M88357
ZNF48	2 (2)	3	2	2	M88358
ZNF49	7 (5)	2	1	3	M88359
ZNF50	3 (1)	2	1	2	M88360
ZNF51	3 (3)	2	1	1	M88362
ZNF52	24 (16)	4	3	15	M88361
ZNF53[f]	1	2	1	2	M88363
ZNF54[f]	1	1	1	1	M88364
ZNF55	1	1	1	2	M88365
ZNF56	1	1	1	2	M88366
ZNF57	3 (3)	1	1	3	M88368
ZNF58	1	2	1	3	M88367
ZNF60	1	1	1	2	M88369
ZNF61	5 (4)	2	1	3	M88370
ZNF62	1	2	1	2	M88371
ZNF64	7 (6)	2	1	3	M88373
ZNF65	10 (7)	3	3	4	M88374
ZNF66	3 (1)	1	1	2	M88375
ZNF67	1	2	1	2	M88376

Note. Genomic clones were sorted into groups that correspond to genes (or loci) that are listed.

[a] The number of clones that occur in each group. The minimum number of different (overlapping) clones in a group was determined by examination of restriction digests. The remaining clones are presumably reisolates of identical clones, but could represent additional overlapping sequences since the restriction analysis was not extensive.

[b] This shows the number of different oligonucleotides that were derived from a particular locus and hybridized to the set of all 208 clones.

[c] this indicates the number of PCR primer pairs derived from sequences of clones of each locus and used on all clones that gave positive signals with oligodeoxynucleotide hybridization.

[d] Number of finger domains sequenced. This number is likely to be lower than the number of finger domains in each gene and may be lower than the fingers listed in Fig. 1.

[e] The characterization of these groups of clones was described in a previous publication (Bray *et al.*, 1991). The cDNA designations are Kox4 for ZNF7, Kox12 for ZNF19, Kox15 for ZNF22, and Kox7 for ZNF44. They are included here for completeness.

[f] ZNF53 and ZNF54 represent two sites each; see Table 2.

described consensus sequence for C_2–H_2 fingers (Schuh *et al.*, 1986). Although the nucleic acid sequences within the finger domains are related, a sufficient number of nucleotide differences occur so that suitably designed oligonucleotide primers are locus-specific and give a band of predicted size when used with cloned DNA templates (Bray *et al.*, 1991). All phage clones tentatively assigned to the same locus were tested as templates for amplification of specific sized DNA fragments using locus-specific oligonucleotide primers; those that produced identical fragments were considered to have been derived from the same locus.

Therefore, partial sequence information verified that each of the 23 loci belong to the zinc finger family and provided an unambiguous identification of each locus by design of primers to be used as STS markers (Fig. 1). Many of these primer pairs have also been used to amplify fragments with genomic DNA as template. These primer pairs provide an easy means of identification of cross-matching clones and can serve also as STS markers to locate these sequences on finer genetic and physical maps. The primer pair sequences, annealing conditions, and expected fragment sizes for each locus are listed in Table 1.

The ZNF52 locus contains 24 clones, while the other loci average 3 clones per group. Although ZNF52 clones cannot be differentiated by the methods used for sorting, the clones show heterogeneity in restriction patterns. Nevertheless ZNF52 clones identify a single map location by hybridization to metaphase chromosomes. In addition, PCR analysis of human/hamster cell line DNA samples (BIOS Corp.), using ZNF52 primer pairs (Table 1), yields appropriate-sized fragments only from human chromosome 3-containing cell lines (data not shown). Thus ZNF52 may represent a complex locus.

In Situ Mapping to Metaphase Chromosomes

One or more clones for each locus were mapped by *in situ* hybridization to human metaphase chromosomes. *In situ* suppression hybridization with biotinylated

ZNF47

ATCCACACCGGCGAGAAGCCGTACAGGTGCGGCCAGTGCGGGAAGTCCTTCATCAAGAAC
I H T G E K P Y R C G Q C G K S F I K N

TCCTCCCTCACTGTGCACCAGCGGATCCACACGGGCGAGAAGCCCTACCGATGCGGCGAG
S S L T V H Q R I H T G E K P Y R C G E

TGCGGGAAGACCTTCAGCCGCAACACGAACCTGACGCGCCACCTGCGGATTCACAC
C G K T F S R N T N L T R H L R I H

ZNF48

GATCTGGTGAAACACCAGCGGACCCACACAGGGGAGAAACCCTACAAGTGTGGGGTCTGT
D L V K H Q R T H T G E K P Y K C G V C

GGCAAGGGCTTTGGGGATAGCTCTGCCCGGATCAAACaCCAGCGGACTCATAGTGGGGAG
G K G F G D S S A R I K H Q R T H S G E

AAGCCTATAGAGCCCGGCCACCAGCCCAGGGTCCCCCAAAGATTCCTCGGT
K P I E P G H Q P R V P Q R F L G

ZNF49

ATCCACACTGGAGAGAAGCCTCACAAATGTAATGAGTGTGGAAAAAGTTTCTGCCGTCTC
I H T G E K P H K C N E C G K S F C R L

TCTCACCTAATCCAACACCAAAGGACCCACAGTGGTGAGAAACCCTATGAGTGTGAGGAG
S H L I Q H Q R T H S G E K P Y E C E E

TGTGGGAAAAGCTTCAGCCGGAGCTCTCATCTAGCTCAGCACCAGAGGACCCACACGGGT
C G K S F S R S S H L A Q H Q R T H T G

GAGAAACCTTATGAATGTAACGAATGTGGCCGAGGCTTC
E K P Y E C N E C G R G F

ZNF50

CACCAGCGGGTGCACACGGGGGAGCGGCCCTACGAGTGTGACGCGTGTGGGAAAGCCTTC
H Q R V H T G E R P Y E C D A C G K A F

AGCCAGAGCACGCACCTGACTCAACACCAGCGCATCCACACCGGGGAGAAGCCCTACAAG
S Q S T H L T Q H Q R I H T G E K P Y K

TGTGACGCGTGTGGCCGAGCCTTCAGCGACTGCTCAGCCCTGATCCGACATCTGAGAATC
C D A C G R A F S D C S A L I R H L R I

CACTCTGGAGAGAAGCCATATCAGTGTAAGGTTTGTCCGAAGGCCTTTGCACAGAGCTCC
H S G E K P Y Q C K V C P K A F A Q S S

TCCCTCATTGAGCACCAGAGGATC
S L I E H Q R I

ZNF51

AAGACCTCTCATGCCTTCTTCTTCGTTCAGGTGGCCCACCTCCGTGCCCATGTGCTTATC
K T S H A F F F V Q V A H L R A H V L I

CACACTGGTGAGAAGCCCTATCCCTGTGAAATCTGTGGCAACCGTTTCCGGCACCTTCAG
H T G E K P Y P C E I C G N R F R H L Q

ACTCTGAAGAGCCACCTGCGAATCCACACAGGAGAGAAACCTTACC
T L K S H L R I H T G E K P Y

ZNF52

TGTAATGAATGTGGGAAAGCCTTTAATCAGAGTGCATGTCTCATGCAGCATCAGAGAATT
C N E C G K A F N Q S A C L M Q H Q R I

CATTCAGGAGAGAAGCCCTACACATGCACTGAATGTGGTAAAGCCTTCACTCAGAACTCT
H S G E K P Y T C T E C G K A F T Q N S

TCCCTTGTTGAACATGAAAGGACTCACACTGGAGAG
S L V E H E R T H T G E

ZNF53

ATTCATACTGGAGAGAAGCCCTATGAATGTAGTAACTGTAGAAAAGCCTTCAGTCACAAG
I H T G E K P Y E C S N C R K A F S H K

GAAAAACTTATTAAACATTATAAAATTCACAGTAGGGAGCAGTCTTACAAATGTAATGAA
E K L I K H Y K I H S R E Q S Y K C N E

TGTGGTAAAGCTTTCATTAAAATGTCAAATCTCATTAGACATCAAAGAATTC
C G K A F I K M S N L I R H Q R I

ZNF54

GCCTCAATCCTTCAAATGCATGCTGGGACTCACCCTGAAGAGAAGCCCTACGAGTGTAAG
A S I L Q M H A G T H P E E K P Y E C K

CAATGTGGGAAAGCCTTCAGATCCTCTAGAGTCGACCTG
Q C G K A F R S S R V D L

ZNF55

ATTCACACTGGAGAGAAACCCTATACATGTAAACAGTGTGGGAAAGCGGTTTTGTCATCT
I H T G E K P Y T C K Q C G K A V L S S

TGGAAGCTTTCAAGACACATGATAATGCACAGTGGAGATGGACCTCATAAATGTAAGATA
W K L S R H M I M H S G D G P H K C K I

TGTGGGAAAGGCTTTGATTTCACGGTTCAGCACGAATTC
C G K G F D F T V Q H E F

FIG. 1. DNA and predicted amino acid sequences of zinc finger domains identified in each of the 23 different loci, with the exception of ZNF7, 19, 22, and 44, which are previously published (Bray *et al.*, 1991).

phage DNA resulted in high signal to noise ratios, greatly reducing the need of a statistical analysis of the mapping data. For each probe, a minimum of 25 metaphase spreads were evaluated, and at least 80% showed specific signals above background fluorescence on both chromatids of both homologs. Two probes, ZNF53 and ZNF54, exhibited more than one signal site (see Table 2), which most likely can be attributed to partial sequence homology with other zinc finger loci.

Chromosome assignment was generally achieved by DAPI banding; however, in a number of cases, chromosome identification was confirmed by cohybridizing with DNA probes specific for the chromosome in question. This was particularly important in the case of chromosome 19, which cannot always be easily distinguished from chromosome 20 after staining with DAPI. In addition, an R-banding pattern generated by hybridization with an *Alu*-DNA clone was used for the band assignment of several probes. A summary of the clones used, their locus number, the chromosomal map position of each locus, and the method used for chromosome identification are given in Table 2. Examples of the mapping data obtained by single and cohybridization experiments are shown in Fig. 2. A summary of the mapping data is illustrated on a banded karyotype idiogram (Fig. 3).

The chromosomal location of the zinc finger loci has been defined as follows. When probes mapped to telomeres, they are listed as mapping to pter or qter. In this case, such coordinates seem more suitable than relating the hybridization signal to distal chromosomal bands or defining them by the fraction of the chromosome length represented by the distance between a chromosomal end and the signal (e.g., FLpter) (Lichter *et al.*, 1990b). The telomeric repeat sequences $(TTAGGG)_n$ visualized by *in situ* hybridization (Moyzis *et al.*, 1988) are usually not seen at the very ends of the chromatids of a metaphase chromosome when the chromosomes are DAPI or propidium iodide counterstained. The zinc finger loci mapped to pter or qter exhibit a chromosomal location that is indistinguishable from that of a telomere repeat probe; therefore it seems more appropriate to simply imply their proximity to telomeric sequences. In contrast, when probes mapped to nontelomeric regions, the subchromosomal localization is given in terms of chromosomal bands. In the case of chromosome 19, the map positions were determined also as FLpter values (data not shown).

```
ZNF56

ATACGACATACTGGAAAAAAATCTTTCAAATGTATAGTATGCGGCAAAGCTTTTAACTCG
I  R  H  T  G  K  K  S  F  K  C  I  V  C  G  K  A  F  N  S

TCCTCAAACCTTACTACACATAAGAAAATTCATACTGGAGAGAAACCCTACAGATGTGAA
S  S  N  L  T  T  H  K  K  I  H  T  G  E  K  P  Y  R  C  E

GAATGTGGCAAAGCCTTCAAAAGGTCCTCACACCTTACTGTACATAAGATAGTTCATACT
E  C  G  K  A  F  K  R  S  S  H  L  T  V  H  K  I  V  H  T

GGAGAGAAATCCTACAAATGTGAAGAATGCGGGAAAGCCTTT
G  E  K  S  Y  K  C  E  E  C  G  K  A  F

ZNF57

GCTGGAGAAAAACCATATGAATGCACCAAGTGCAGGACAGTCTTCACGCATCTTTCTTCT
A  G  E  K  P  Y  E  C  T  K  C  R  T  V  F  T  H  L  S  S

CTTAAAAGGCACGTCAAGTCTCACTGTGGACGAAAAGCACCTCCAGGTGAGGAATGT
L  K  R  H  V  K  S  H  C  G  R  K  A  P  P  G  E  E  C

ZNF58

CTGCAGCCCACTTCCAGTTCCCTTCGAAAACATGAAACAACACACACTGGAGAGCAACCC
L  Q  P  T  S  S  S  L  R  K  H  E  T  T  H  T  G  E  Q  P

TATAAATGTAAATGTGGAAAAGCTTTTAGTGATTTATTTTCCTTTCAAAGTCATGAAACA
Y  K  C  K  C  G  K  A  F  S  D  L  F  S  F  Q  S  H  E  T

ACACACAGTGAAGAGGAGCCTTATGAATGTAAGGAGTGTGGGAAAGCATTTAGTTCTTTT
T  H  S  E  E  E  P  Y  E  C  K  E  C  G  K  A  F  S  S  F

AAATACTTTTGTCGCCATGAAAGGACTCACAGTGAAGAAAAATCTTATGAGTGTCAAATT
K  Y  F  C  R  H  E  R  T  H  S  E  E  K  S  Y  E  C  Q  I

TGTGGCCAAAGCTTCAGTCG
C  G  Q  S  F  S

ZNF60

ATCCACACGGGGGAGAAGCCCTTCTCGTGCCGGGAGTGCAGCAAGGCC
I  H  T  G  E  K  P  F  S  C  R  E  C  S  K  A

ZNF61

GAGAGAAAGCCCTATGGATGCAACGAGTGTGGGAAAACCTTCAGCCACAGCTCCTCACTC
E  R  K  P  Y  G  C  N  E  C  G  K  T  F  S  H  S  S  S  L

AGCCAGCATGAGCGGACACACACAGGAGAGAAGCCCTATGAGTGCAGTCAGTGTGGGAAG
S  Q  H  E  R  T  H  T  G  E  K  P  Y  E  C  S  Q  C  G  K

GCCTTCCGGCAGAGCACACACCTCACCCAACACCAGCGAATCCACACAGGGGAGAAGCCC
A  F  R  Q  S  T  H  L  T  Q  H  Q  R  I  H  T  G  E  K  P

TATGAATGCAATGACTGCGGCAAGGCATTCAGTCACAGCTCGTCCCTCACCAAACATCAG
Y  E  C  N  D  C  G  K  A  F  S  H  S  S  S  L  T  K  H  Q

CGAATCCACACTGGGGAGAAGCCCTACGAATGCAACCAGTGT
R  I  H  T  G  E  K  P  Y  E  C  N  Q  C

ZNF62

ATCCACACCGGGGAGAAGCCCTACCCTTGCCCTACCTGCGGAACCCGCTTCCGCCACCTG
I  H  T  G  E  K  P  Y  P  C  P  T  C  G  T  R  F  R  H  L

CAGACCCTCAAGAGCCACGTTCGCATCCACACCGGAGAGAAGCCTTAC
Q  T  L  K  S  H  V  R  I  H  T  G  E  K  P  Y

ZNF64

TCCCGACTCATTTACCACCAGAGAACCCATACTCGGGAGAAACCTTATGAATGCAATGCG
S  R  L  I  Y  H  Q  R  T  H  T  R  E  K  P  Y  E  C  N  A

TGTGGAGAGGCATTCATTCGAAGCAAAAGTCTTGCTCGACATCAGGTCCTGCACACTGGT
C  G  E  A  F  I  R  S  K  S  L  A  R  H  Q  V  L  H  T  G

AAGAAACCTTACAAATGCAATGCGTGTGGGAGAGCATTCTGTTCCAATAGAAATCTCATT
K  K  P  Y  K  C  N  A  C  G  R  A  F  C  S  N  R  N  L  I

GACCATCAGAGAATCCACACTtGGGAGAAGCCTTATGAGTGTAGTGAATGTGGCAAAGCC
D  H  Q  R  I  H  T  W  E  K  P  Y  E  C  S  E  C  G  K  A

TTCAGTCGGAGTAAATGTCTTATTCGACATCAGAGCCTCCATACTGGGGAAAAGCCATAC
F  S  R  S  K  C  L  I  R  H  Q  S  L  H  T  G  E  K  P  Y

AAATGT
K  C

ZNF65

CTGGAGAAGCCCTACATCTGCAACGAGTGCGGCAAGAGCTTCAGCCAGTGGTCCAAGCTG
L  E  K  P  Y  I  C  N  E  C  G  K  S  F  S  Q  W  S  K  L

CTGCGGCACCAGCGCATCCACACGGGAGAGCGGCCCAACACCTGCTCCGAGTGCGGCAAG
L  R  H  Q  R  I  H  T  G  E  R  P  N  T  C  S  E  C  G  K

AGCTTCACGCAGAGCTCGCACCTGGTGCAGCACCAGCGCACGCACACCGGCGAGAAGCCC
S  F  T  Q  S  S  H  L  V  Q  H  Q  R  T  H  T  G  E  K  P

TACAAGTGCCCCGACTGCGGCAAGTGCTTCAGCTGGAGCTCCAACCTGGTGCAGCACCAG
Y  K  C  P  D  C  G  K  C  F  S  W  S  S  N  L  V  Q  H  Q

CGCACGCACACGGGAGAGAAGCCCTACAAGTGCACGGAGTGCGAGAAAGCCTTCACCCAG
R  T  H  T  G  E  K  P  Y  K  C  T  E  C  E  K  A  F  T  Q

AGCACCAACCTCATCAAGCACCAGCGATC
S  T  N  L  I  K  H  Q  R

ZNF66

CTTTCTTCCCTTTCTACACATAAGATAATTCATACTGGAGAGAAACCCTACAAATGTGAA
L  S  S  L  S  T  H  K  I  I  H  T  G  E  K  P  Y  K  C  E

GAATGTGGTAAAGCCTTCAACTGGTCCTCACACCTTACTACACATAAGAGAATTCATACT
E  C  G  K  A  F  N  W  S  S  H  L  T  T  H  K  R  I  H  T

GGAGAGAAACCCTACAAATGTGAAGAATGTGGCAAAAGCTTTAAG
G  E  K  P  Y  K  C  E  E  C  G  K  S  F  K

ZNF67

ATTCATACTAGAGGGAAACATTACAAGTGTGAAGAAAGTGGCAAAGCATTTATCTGGTCC
I  H  T  R  G  K  H  Y  K  C  E  E  S  G  K  A  F  I  W  S

TCAGGCCTTACTGAACATAGGAGAGTTCATACTAGACAGAAACCCTACAAATGTGAAGAA
S  G  L  T  E  H  R  R  V  H  T  R  Q  K  P  Y  K  C  E  E

TGTGGCAAAGCATTAATTCAATTCTCAACCCTAACTAGACATAAGAGGATACACACTGGA
C  G  K  A  L  I  Q  F  S  T  L  T  R  H  K  R  I  H  T  G

GAGAAACCC
E  K  P
```

FIG. 1—*Continued*

Several striking features of the chromosomal localization of this subset of zinc finger encoding sequences are apparent from the data given in Table 2. (i) Many of the loci (10 out of 23) are in the telomeric regions of chromosomes and (ii) many loci are clustered (see Fig. 3, chromosomes 1, 16, and 19). Interestingly, 10 out of 23 probes map on human chromosome 19. With the exception of ZNF58 and a secondary site of ZNF53, the 9 other loci on chromosome 19 fall into three clusters. (iii) Ten of the zinc finger loci are associated with the cytogenetic bands that can exhibit chromosome fragility. Fragile sites are regions that express gaps or discontinuities on one or both chromatids under appropriate conditions. Although the fragile site at Xq27 is clearly implicated in mental retardation, phenotypes have not been described for any of the other fragile sites so far identified (Sutherland, 1991), and the molecular properties of these sites are still unknown.

DISCUSSION

We have identified 23 loci in the human genome encoding zinc finger motifs and determined their chromosomal localization. These genes have been isolated by their sequence homology to the H/C link region of the Drosophila segmentation gene Krüppel and thus represent members of a large subclass of zinc finger genes. Based on this relationship with the Krüppel gene, it is possible that some of the human genes isolated here are involved in developmentally important regulatory func-

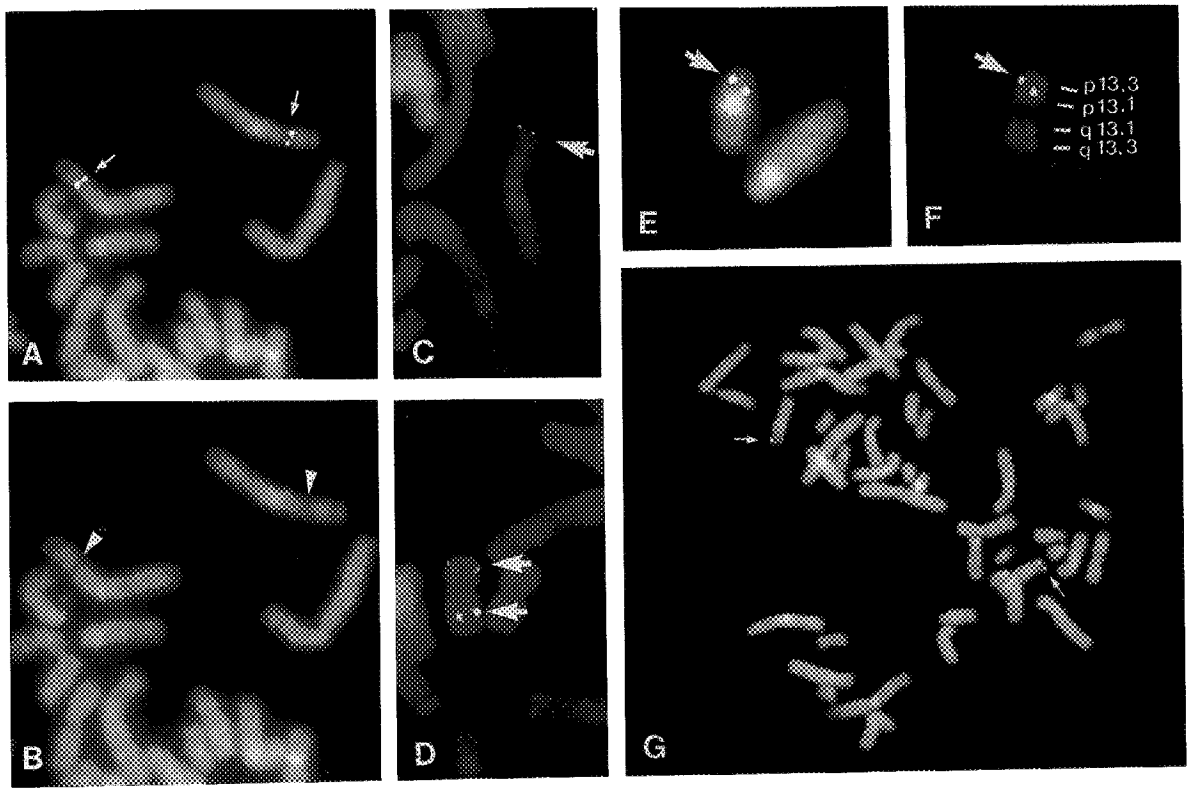

FIG. 2. *In situ* hybridization mapping of genomic DNA probes encoding zinc finger motifs. Probes were biotinylated, hybridized to human chromosomes under suppression hybridization conditions, and detected via avidin–FITC (see arrows). Chromosomes were counterstained or banded with DAPI (**A, B, E,** and **G**), PI (C and D), or by cohybridization with an *Alu*-DNA probe (**F**). Digital images were taken separately for each fluorochrome color, and two-color images were obtained by electronic overlay of two images (**A, C–G**). (**A**) Highly specific signals of clone 7 (ZNF52) on the short arm of chromosome 3. Localization within 3p21 is demonstrated by DAPI banding (compare **A** with **B**, where the location of the signal in **A** is depicted by arrowheads). (**C**) Probe 238 mapping to the terminal region of 11p. (**D**) Probe 111 (ZNF53) mapping to 19pter and to the long arm of chromosome 19. (**E**) Probe 8 (ZNF58) localized on counterstained chromosome 19. (**F**) Same chromosomes as in **E** but after *Alu* banding, showing the location of ZNF58 in 19p13.2 (note that the *Alu* sequence does not label the centromeric region). (**G**) Non-zinc-finger encoding subfragment of clone 36 mapped to 12qter, the same position as the clone36, showing that the mapping is not based on a homology between the zinc finger motif DNA sequence and a telomeric sequence.

tions. A number of these loci cluster in the same chromosomal region, notably in 16p11, 19pter, 19p13.1, and 19qter. A disproportionate abundance of zinc finger genes on Chr 19 has also been observed by other workers (Huebner *et al.*, 1991; Hoovers *et al.*, 1992). Hoovers and colleagues have also identified six cosmids that contain the zinc finger motif and map to chromosome 3p21. It is possible that some of the clones identified by Hoovers *et al.* on chromosomes 19 and 3 are identical to those presented in this paper; we are cross-matching the cosmids using the locus-specific primer pairs presented here for PCR analysis.

Whether the presence of two or more zinc finger genes at the same cytogenetic location represents a functional finger gene cluster cannot be determined at present since the resolution of *in situ* hybridization to metaphase chromosomes is not sufficiently high. Thus, an apparent cluster could be constituted by several finger genes distributed over a region of a few megabases and could correspond to entirely distinct loci with independent regulatory pathways. Alternatively, loci found at the same location might be more tightly clustered and could form a complex locus with functional and regulatory relationships between the individual genes. Although members of gene families that are expressed in a cell type-specific fashion frequently are found widely distributed over the genome (e.g., cardiac versus skeletal muscle myosin heavy chain genes, the actin genes, or the myosin light chain genes; see McAlpine *et al.*, 1990), developmentally regulated members of a multigene family expressed sequentially during development often cluster within a particular genomic region (e.g., α and β hemoglobin genes, HOX class homeobox genes, interleukin genes, and interferon genes). The HOX genes are a particularly interesting example; while the genes in a cluster are not activated at the same time and place during development, the relationship of chromosomal arrangement, temporal sequence of activation, and spatial order of expression is conserved in species as widely diverged as Drosophila and mammals (reviewed by De Robertis *et al.*, 1990). It will be interesting to test whether zinc finger genes form functional clusters with similar properties.

The zinc finger loci presented here have a biased chromosomal localization (see Table 2). Of the 23 loci, 10 map to the telomeric region of a human chromosome arm, and many of the loci not located in the telomeric region are in the area of chromosomal fragile sites. Al-

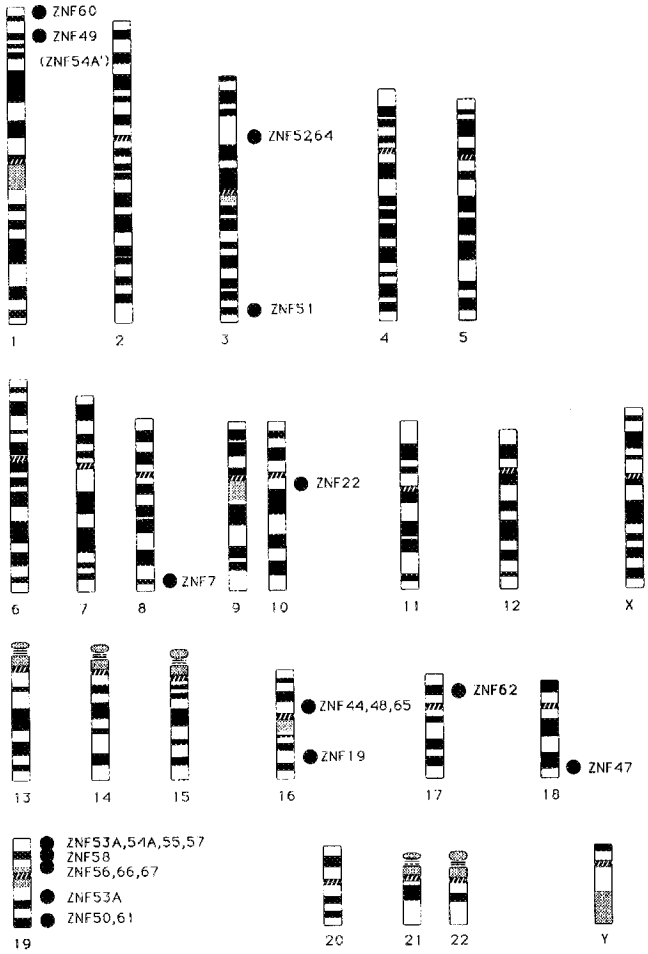

FIG. 3. Idiogram illustrating the distribution of zinc finger loci on human chromosomes.

though not all chromosomal fragile sites are mapped to a narrow chromosomal band, and in some cases the adjacent fragile site is proximal or distal to the mapped zinc finger containing locus (Table 2, last column), the correlation is intriguing. We do not feel that this reflects the abundance of both fragile sites and zinc finger genes within the genome since most of the fragile sites that have been identified are interstitial (Sutherland, 1991) and few are at telomeres, whereas the majority of zinc finger loci described here map to telomeres. However, because the spatial resolution of mapping on metaphase chromosomes is in the 1-Mb range, it will be necessary to carry out additional fine mapping on interphase chromosomes to address the possible colocalization of fragile sites and zinc finger loci.

A recent analysis of telomere-related sequences shows that telomeric repeat sequences are present at interstitial sites in the human genome (Wells *et al.*, 1990). In particular, a telomeric sequence that has been identified at 2q13–q14 is considered the fusion point of two ancestral ape chromosomes (IJdo *et al.*, 1991). This telomeric sequence maps close to a known fragile site (IJdo *et al.*, 1992). Fragile sites may therefore be chromosomal regions that were located at telomeres in prior times but were rearranged during the chromosomal evolution of a species and may be both reminiscent of and predisposing for evolutionary chromosomal rearrangements.

One question raised by the biased distribution of zinc finger sequences at telomeres of human chromosomes is whether this localization affects the transcription of these genes; if this were the case, selection pressure might maintain the localization of these genes in telomeric regions. A localization of the telomeres in the periphery of the nucleus, where gene expression appears to be predominantly localized (Blobel, 1985; Hutchison and Weintraub, 1985), has been proposed (Meyne *et al.*, 1990; Rabl, 1885). While a peripheral localization of expressed genes may not be generally true (Manuelidis and Borden, 1988; Spector, 1990) it might be functionally important for some classes of genes. This might be especially relevant in rapidly dividing embryonic cells in which it might be advantageous to have the genes located close to the nuclear membrane to facilitate the export of nascent RNA. Thus, the colocalization of telomeres and zinc finger encoding genes might reflect selection pressure due to the three-dimensional organization of the corresponding genes in the cell nucleus.

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